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(54) Title: GENE CONTROLLING FLORAL DEVELOPMENT AND APICAL DOMINANCE IN PLANTS

(57) Abstract

The present invention provides compositions and methods for affecting the transition from vegetative to reproductive growth in a wide variety of plants. A MADS-box gene from rice, OsMADS1, has been isolated and sequenced. Expression of OsMADS1 in transgenic plants dramatically alters development, resulting in early flowering plants with reduced apical dominance, causing both long-day and short-day plants to flower under both short-day and long-day conditions. OsMADS1 is a key regulatory factor determining the transition from shoot apex to floral meristem and is a target for action of flower induction signals.

GENE CONTROLLING FLORAL DEVELOPMENT AND APICAL DOMINANCE IN PLANTS

TECHNICAL FIELD

This invention is related to the field of compositions and methods for
affecting plant floral development and more particularly to an isolated gene and its use
in affecting the timing of the transition from vegetative to reproductive growth.

BACKGROUND ART

A majority of plants use environmental cues to regulate the timing of the
transition from vegetative to reproductive growth in order to ensure synchronous
flowering for successful outcrossing and to complete their sexual reproduction under
favorable conditions (reviewed in Bernier et al., Plant Cell 5:1147-1155, 1993). The
major environmental factors that control the transition are photoperiod, temperature,
and nutrition.

In responding to these environmental factors, plants differ widely among
species, among cultivars within species, and among stages of plant development
within a cultivar. A short-day plant flowers when the day length is less than its critical
length and a long-day plant flowers when the day length is longer than its critical
length. Floral induction in a day-neutral species is unaffected by day-length, but
occurs when the plant has attained a minimum amount of growth.

After completion of the basic vegetative phase, initiation of flowering is
frequently dependent on the day length. The critical photoperiod is defined as the
maximum day length that will induce a short-day plant to flower and the minimum day
length that will induce a long-day plant to flower.

It has been postulated that a transmissible flowering signal is produced
mainly in leaves and is transported to the shoot apex through the phloem. Grafting
experiments have shown that leaves of photoperiodic plants produce promoters and
inhibitors of flowering when exposed to favorable and unfavorable daylength regimes,
respectively (Lang et al., Proc. Natl. Acad. Sci. USA 74:2412-2416, 1977). The
nature of these transmissible signals is still controversial (O'Neill, Photochem.
Photobiol. 56:789-801, 1992) and efforts to isolate the signaling substances have
been unsuccessful. In addition, the target genes for these signals in the shoot apex
have not been identified.

Significant effort has been expended in attempts to elucidate the
underlying mechanisms controlling flower development in various dicotyledonous plant
species (reviewed in Coen, Annu. Rev. Plant Physiol. Plant Mol. Biol. 42:241-279,
1991; and Gasser, Annu. Rev. Plant Physiol. Plant Mol. Biol. 42:621-649, 1991),
leading to the isolation of a family of genes which encode regulatory proteins. These

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regulat ry proteins include *AGAMOUS* (*AG*) (Yanofsky et al., Nature 346:35-39, 1990), *APETELA I* (*API*) (Mandel et al., Nature 360:273-277, 1992), and *APETALA 3* (*AP3*) (Jack et al., Cell 68:683-697, 1992) in *Arabidopsis thaliana*, and *DEFICIENS A* (*DEF A*) (Sommer et al., EMBO J. 11:251-263, 1990), *GLOBOSA* (*GLO*) (Trobner et al., EMBO J. 11:4693-4704, 1992), *SQUAMOSA* (*SQUA*) (Huijser et al., EMBO J. 11:1239-1249, 1992), and *PLENA* (*PLE*) (Bradley et al., Cell 72:85-95, 1993) in *Antirrhinum majus*.

Mutations in an *AG* or *PLE* gene result in homeotic alterations of the stamen and carpel. Genetic studies have shown that the *DEF A*, *GLO* and *AP3* genes are essential for petal and stamen development. *API* and *SQUA* genes, which are expressed in young flower primordia, are necessary for the transition of an inflorescence meristem into a floral meristem. Sequence analysis of these genes has revealed that their gene products contain a conserved MADS-box region (Bradley et al., Cell 72:85-95, 1993; Huijser et al., EMBO J. 11:1239-1249, 1992; Jack et al., Cell 68:683-697, 1992; Mandel et al., Nature 360:273-277, 1992; Sommer et al., EMBO J. 11:251-263, 1990; Trobner et al., EMBO J. 11:4693-4704, 1992; Yanofsky et al., Nature 346:35-39, 1990), which is probably a DNA-binding domain (Schwarz-Sommer et al., EMBO J. 11:251-263, 1992).

Using these clones as probes, MADS-box genes have been isolated from other species including tomato (Mandel et al., Cell 71:133-143, 1992), tobacco (Kempin et al., Plant Physiol 103:1041-1046, 1993), petunia (Angenent et al., Plant Cell 4:983-993, 1992), *Brassica napus* (Mandel et al., Cell 71:133-143, 1992), and maize (Schmidt et al., Plant Cell 5:729-737, 1993).

Transgenic approaches have been employed to study the functional roles of MADS-box genes. Genetic complementation of the *ag-2* mutant by the *AG* gene demonstrated that the *ag-2* gene product is involved in stamen and carpel development (Yanofsky et al., Nature 346:35-39, 1990). Ectopic expression of the *AG* genes from *A. thaliana*, *B. napus*, petunia, tobacco, and tomato resulted in homeotic conversion of sepals to carpels and petals to stamens, mirroring the *ap2* mutant phenotype (Kempin et al., Plant Physiol 103:1041-1046, 1993; Mandel et al., Cell 71:133-143, 1992; Mizukami and Ma, Cell 71:119-131, 1992; Pnueli et al., Plant Cell 6:163-173, 1994; Tsuchimoto et al., Plant Cell 5:843-853, 1993). These results support the hypothesis that *AG* and *AP2* act in an antagonistic fashion.

Antisense approaches have also been used to reveal the functional role of the tomato MADS-box genes (Pnueli et al., Plant Cell 6:175-186, 1994; Pnueli et al., Plant Cell 6:163-173, 1994). Transgenic plants that express tomato *AG* antisense RNA display the *ag* mutant phenotypes. Antisense expression of the tomato *TM5*

MADS-b x gene results in morphological changes in the three inner wh rls of transgenic plants.

The timing of the transition from vegetative growth to flowering is one of the most important steps in plant development. This determines quality and quantity of most crop species since the transition determines the balance between vegetative and reproductive growth. It would therefore be highly desirable to have means to affect the timing of this transition. The present invention meets this and other needs.

SUMMARY OF THE INVENTION

The present invention provides compositions and methods related to the OsMADS1 gene of *Oriza sativa* and homologs thereof, such as the NtMADS1 gene of *Nicotiana tabacum* and their use to alter the phenotype of a plant, including phenotypes related to the timing of the transition between vegetative and reproductive growth.

It is an object of the invention to provide isolated nucleic acids comprising at least 15 contiguous nucleic acids of (1) a native OsMADS1 gene, including alleles and homologs or (2) variants of a native OsMADS1 gene, and preferably the full length native sequence of OsMADS1 or NtMADS3 as shown in Figs. 1A or 10, respectively. When expressed in a transgenic plant, the OsMADS1 gene produces at least one phenotype including (1) diminished apical dominance, (2) early flowering, (3) a partially or completely altered daylength requirement for flowering, (4) greater synchronization of flowering, or (5) a relaxed vernalization requirement. Variant forms of the OsMADS1 gene are preferably substantially similar in sequence to a native OsMADS1 gene and preferably comprise only silent or conservative substitutions to a native OsMADS1 gene sequence.

A further object of the invention is to provide host cells and transgenic plants in which an OsMADS1 gene or fragment thereof is introduced.

A further object of the invention is to provide purified polypeptides encoded by an OsMADS1 nucleic acid, e.g., by expression of an expression vector comprising an OsMADS1 gene or fragment thereof in a suitable host cell.

A further object of the invention is to provide probes and primers comprising a fragment of an OsMADS1 gene capable of hybridizing under stringent conditions to a native OsMADS1 gene sequence. Such probes and primers are useful, for example, in studying the structure and function of OsMADS1 genes and for obtaining homologs of the OsMADS1 gene from plants other than rice and tobacco.

The expressi n of an OsMADS1 transgene in transgenic plants of a long-day or short-day species, as noted above, can result in day-neutral phen type, for example. Therefore, it is a further object of the invention t use such transgenic

plants t pr duce multiple crops in a year, which therwise cannot be accomplish d under natural photoperiod conditions.

The foregoing and other objects and advantages of the invention will become more apparent from the following detailed description and accompanying

5   drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1A shows the nucleotide and deduced amino acid sequences of OsMADS1 cDNA. MADS-box and K-box regions are underlined. The positions of nucleotides and amino acids are shown on the left and right, respectively.

10   Fig. 1B provides a comparison of MADS-box regions, showing the alignment of the MADS-box sequence of OsMADS1 (residues 2-57) with the MADS-box sequence of AP1, SQUA, AG, PLE, AP3, DEF A. The asterisks indicate amino acids that are identical to corresponding amino acids of OsMADS1.

Figs. 2A and 2B show the results of a Southern blot analysis of rice DNA

15   digested with EcoRI (E), HindIII (H), or PstI (P) and hybridized with a probe prepared from the entire OsMADS1 cDNA (Fig. 3A) or an OsMADS1 cDNA probe lacking the conserved MADS-box region (Fig. 3B). The position of HindIII-digested lambda DNA size markers is also indicated in both Figs. 2A and 2B.

Figs. 3A and 3B show the results of a Northern blot analysis of the

20   OsMADS1 transcript in rice, using as a probe a OsMADS1 cDNA lacking the MADS domain. Fig. 3A specifically shows OsMADS1 expression patterns in rice organs: (L) leaf, (R) root, (A) anther, (C) carpel, and (P) palea/lemma of anthesis-stage flowers. Fig. 3B shows a temporal expression pattern during flower development, wherein total RNA isolated from rice flowers at different developmental stages was used for

25   detection of OsMADS1 gene expression: 1, young inflorescence (panicle size < 1 cm); 2, young flower (panicle size = 1 to 6 cm); 3, flower at the early vacuolated pollen stage; and 4, flower at the late vacuolated pollen stage.

Figs. 4A - 4E depict localization of the OsMADS1 transcript in rice flowers and phenotypes of transgenic tobacco plants expressing OsMADS1, wherein

30   Fig. 4A shows a young inflorescence; Figs. 4B and 4C show cross sections of upper and lower rice flowers at the vacuolated pollen stage, respectively (A, anther; F, filament; FP, flower primordia; L, lemma; O, ovary; P, palea; S, sheath; SL, sterile lemma); Fig. 4D provides a comparison of OsMADS1 transgenic plant #7 (left) and a wild-type SR1 tobacco (right); and Fig. 4E is a close-up picture of OsMADS1

35   transgenic plant #2 showing branching and bushy phenotypes.

Fig. 5 shows the results of a northern blot analysis of OsMADS1 transcripts in transgenic tobacco, using as the probe an OsMADS1 cDNA lacking the MADS-box domain, wherein the "C" denotes a control plant and the numerals "1"

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through "7" denote s ven diff rent transgenic plants exhibiting the early flowering and dwarf phen types.

Fig. 6 shows phenotypes of transgenic *N. sylvestris* plants expressing OsMADS1. Transgenic (left) and untransformed control (right) plants grown under long-day growth condition (16 hr daylength) (Fig. 6A) or a short-day growth condition (10 hr daylength) (Fig. 6B).

Fig. 7 shows the results of a northern blot analysis of OsMADS1 transcripts in transgenic *N. sylvestris* using as a probe an OsMADS1 cDNA lacking the MADS-box domain. "1" through "5" denote five independently transformed transgenic plants exhibiting early flowering under both permissive and non-permissive conditions. C, control untransformed control *N. sylvestris*.

Fig. 8 shows phenotypes of transgenic *N. tabacum* cv. Maryland Mammoth plants expressing OsMADS1. Transgenic plants (left) and untransformed control (right) grown under a short-day growth condition (10 hr daylength) (Fig. 6A) or a long-day growth condition (16 hr daylength) (Fig. 6B).

Fig. 9 shows the results of a northern blot analysis of OsMADS1 transcripts in transgenic *N. tabacum* cv. Maryland Mammoth using as a probe an OsMADS1 cDNA lacking the MADS-box domain. "1" through "3" denote three independently transformed transgenic plants exhibiting early flowering under both permissive and non-permissive conditions. C, untransformed control *N. tabacum* cv. Maryland Mammoth.

Fig. 10 shows the nucleotide and deduced amino acid sequence of the NtMADS3 cDNA. The positions of nucleotides and amino acids are shown on the left and right, respectively.

Fig. 11 shows a nucleotide sequence comparison of the NtMADS3 cDNA (top) with the OsMADS1 cDNA (bottom). The positions of the nucleotides for each cDNA are shown on the left and right.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides compositions and methods related to a "MADS-box" gene from rice, OsMADS1, and its homologs that are useful, for example, for producing dwarf plants and for affecting the timing of the transition from vegetative to reproductive growth in a wide variety of plants, including various dicotyledonous and monocotyledonous crop plants and tree species (see Schwarz-Sommer et al., Scienc 250:931-936, 1990 regarding "MADS-box" genes).

OsMADS1 is a key regulatory factor determining the transition from shoot apex to floral meristem and is likely a target for action f flower induction signals.

The mechanism for the function of the rice OsMADS1 gene product is unknown. The OsMADS1 gene product may regulate the expression of genes that are

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involved in the induction f fl wering, thus leading to the early fl wering and dwarf phenotypes observed in day-neutral plants and verriding the ph top riod requirement for flowering in flowering plants of a species that is normally daylength-sensitive (i.e., a long-day and short-day species). The OsMADS1 polypeptide may, for example, act as a positive regulatory factor similar to *AP1* or *SQUA*. Since the 35S promoter is active in most cell types, it is likely that OsMADS1 polypeptide produced under the control of the 35S promoter is accumulated in the shoot meristem and inflorescence meristem, where the *AP1* (*SQUA*)-like gene is not yet activated. It has been reported that at least two genes, *AP1* and *LEAFY*, are required for the transition of inflorescence meristem into floral meristem in *Arabidopsis* (Weigel et al., Cell 69:843-859, 1992). Similarly, *SQUA* and *FLORICAULA* are required for floral organ induction in *Antirrhinum majus* (Coen et al., Cell 63:1311-1322, 1990). It was determined that *AP1* and *SQUA* belong to the MADS-box gene family (Coen et al., Cell 63:1311-1322, 1990; Weigel et al., Cell 69:843-859, 1992). If *AP1* is normally expressed later than *LEAFY*, the ectopic expression of OsMADS1 may bypass the transient period required for normal floral organ development. Alternatively, the OsMADS1 protein may interact with a negative factor that normally inhibits flowering. It is also possible that a higher level of expression of OsMADS1 may enhance the response to flower-promoting signals.

No other MADS-box genes have been shown to induce early flowering and dwarfing and to override photoperiod requirements, for example. Although the early flowering and dwarf phenotypes were not apparent in transgenic plants in their juvenile state, axillary bud growth was initiated during the early stage of floral meristem development. The growing shoot apex is known to exert an influence over a range of developmental events, including axillary bud growth (Tamas, Hormonal regulation of apical dominance, In: Davies, ed., Plant Hormones and Their Role in Plant Growth and Development, Martinus Nijhoff Pub., Dordrecht, Netherlands, 1987, pp. 393-410). The effect is greatest early in plant development. As plants mature, the emergence of floral organs releases inhibition of the lateral buds and allows them to develop. The active substance responsible for apical dominance in a number of plant species has been identified as the plant growth regulator, indoleacetic acid (IAA). In transgenic plants expressing OsMADS1, the dwarf phenotype may be the result of altered hormonal status due to early flowering.

Use of the OsMADS1 Gene and its Homologs for Crop Improvement

According to the present invention, the OsMADS1 gene is useful, for th f llowing purposes, among thers. (Reference to the OsMADS1 gene is intended to encompass alleles, homologs, and variants as well).

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Early flowering. The timing f the transition between veg tative and
reproductive growth is an important agr n mic trait, serving as a crucial fact r in
determining crop yields. Expression of the OsMADS1 gene in transgenic plants makes
it possible to affect the transition from vegetative to reproductive growth in a wide

5   variety of plants, whether the plants are long-day, short-day, or day-neutral plants.

When the OsMADS1 gene is expressed in transgenic plants of day-
neutral species, the resulting transgenic plants flower earlier than control plants.
Transgenic long-day and short-day flowering plants expressing the OsMADS1 gene
also flower earlier under permissive conditions than control plants. The compositions

10   and methods according to the present invention therefore permit one to reduce the
length of the vegetative growth stage of cereal, fruit, vegetable, floricultural, and other
crop species.

Producing dwarf plant varieties. Although it has been possible to
enhance the harvest index in grain crops by the use of dwarfing genes, the isolation of

15   these genes producing dwarf phenotypes has been difficult.

Transgenic plants comprising a OsMADS1 transgene are shorter than
controls. Expression of an OsMADS1 transgene apparently causes no significant
change in the rate of photosynthesis or the total mass of the plant. Therefore, the
OsMADS1 gene is useful for producing dwarf plant varieties for a variety of plants

20   including cereal, fruit, and floricultural species.

Synchronizing reproductive growth. Transgenic plants expressing the
OsMADS1 transgene flower more synchronously than controls. Therefore, the gene
can be used for crops for which synchronized harvesting is economically beneficial,
allowing more effective use of mechanized harvesting of fruit species or the

25   production of floricultural species having improved flower quality, for example.

Producing day-neutral plant varieties. Expression of an OsMADS1
transgene in daylength-sensitive (i.e., long-day or short-day) plants at least partially
overrides the photoperiod requirement for flowering and can completely override the
photoperiod requirement. By introducing such a transgene into a wide variety of

30   photoperiod-sensitive crop species, including, but not limited to rice and soybeans,
these plants effectively become day-neutral, permitting multiple crops to be grown per
year. For example, flowers can be induced the year-round by introducing an
OsMADS1 transgene into floricultural species such as chrysanthemum and orchid.

Delaying flowering and fruiting. By suppressing the expression of the

35   OsMADS1 gene by conventional approaches, e.g., by employing antisense, co-
suppression, gene replacement, or ther c nventional approaches to suppressing plant
gene expression, flowering and fruiting can be delayed. Delayed reproductive growth
can thereby increase the length of th vegetative growth stage and cause the plants

to gr w faster, since the energy used f r development of flowers and s eds can be saved for vegetative growth. Thus, delaying or eliminating reproductive gr wth r sults in a higher yield of vegetable species such as spinach, radish, cabbage, or tree species. In addition, such plants will be more desirable for as garden and street species, since their production of pollen allergens can be reduced or eliminated.

Overcoming the vernalization requirement. The OsMADS1 gene is useful for overriding the vernalization requirement of certain plant species. It is likely that the OsMADS1 gene is the last (or almost last) gene expressed in the flower induction pathway. Therefore, induction of flowering of transgenic plants that constitutively express the OsMADS1 gene becomes insensitive to temperature.

Growing plants in space. Plants grown extraterrestrially are preferably insensitive to photoperiod and temperature for flowering. Transgenic plants carrying the OsMADS1 gene would be expected to flower in the extremely abnormal growth conditions found in a space shuttle or space station.

Cloning and analysis of homologs of the OsMADS1 gene in other plants. The availability of the OsMADS1 gene and its sequence makes it possible to obtain homologs of the OsMADS1 gene in other organisms by conventional methods, through the use of nucleic acid and antibody probes and DNA primers based on the OsMADS1 sequence, as described in greater detail below. These probes and primers are also useful in research on the patterns of expression and structure-function relationships for rice OsMADS1 and its homologs, and for determining the interaction of these genes with other plant genes involved in floral development, for example.

DEFINITIONS AND METHODS

The following definitions and methods are provided to better define the present invention and to guide those of ordinary skill in the art in the practice of the present invention. Definitions of common terms in molecular biology may also be found in Rieger et al., Glossary of Genetics: Classical and Molecular, 5th edition, Springer-Verlag: New York, 1991; and Lewin, Genes V, Oxford University Press: New York, 1994.

The term "plant" encompasses any plant and progeny thereof. The term also encompasses parts of plants, including seed, cuttings, tubers, fruit, flowers, etc.

A "reproductive unit" of a plant is any totipotent part or tissue of the plant from which one can obtain a progeny of the plant, including, for example, seeds, cuttings, buds, bulbs, somatic embryos, etc.

"Natural photoperiod conditions" are photoperiod (i.e., daylength) conditions as provided by sunlight at a given location, whether under field conditions. A photoperiod provided by artificial lighting but having a daylength approximating that of sunlight would also be considered a natural photoperiod condition.

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Nucleic Acids

Nucleic acids useful in the practice of the present invention comprise the isolated rice OsMADS1 gene, its homologs in other plant species, and fragments and variants thereof.

5   The term "OsMADS1" gene refers to a plant gene that contains a MADS-box sequence, and preferably also a K-box sequence, and that is associated with one or more of the following phenotypes when expressed as a transgene in transgenic plants: (1) diminished apical dominance (as shown, for example, by dwarf stature); (2) early flowering; (3) altered daylength requirement for flowering; (4)

10   greater synchronization of flowering; and (5) relaxed vernalization requirement. This term relates primarily to an isolated OsMADS1 coding sequence, it also encompasses sequences comprising genomic sequences flanking the OsMADS1 coding sequence that are operably linked to the coding sequence, including regulatory elements and/or intron sequences. Although the term "OsMADS1 gene" most properly applies to the

15   rice OsMADS1 gene as shown in Fig. 1A, it is also intended to encompass alleles of the rice OsMADS1 gene and homologs thereof from other plant species, such as the NtMADS1 gene of *Nicotiana tabacum*, as shown in Fig. 10. The term "OsMADS1 gene" also encompasses "variant" forms of the gene (as detailed below) that have substantial homology to an OsMADS1 sequence and OsMADS1 biological activity.

20   The term "native" refers to a naturally-occurring nucleic acid or polypeptide, including a wild-type sequence and an allele thereof.

A "homolog" of the OsMADS1 gene is a native OsMADS1 gene sequence isolated from a plant species other than *Oriza sativa*. The homolog has at least one of the biologically activities of OsMADS1, e.g., affecting apical dominance in

25   transgenic plants, etc., as discussed above.

The nucleic acids of the present invention can be free in solution or attached by conventional means to a solid support, such as a hybridization membrane (e.g., nitrocellulose or nylon), a bead, or other solid supports known in the art.

"Isolated". An "isolated" nucleic acid has been substantially separated

30   or purified away from other nucleic acid sequences in the cell of the organism in which the nucleic acid naturally occurs, i.e., other chromosomal and extrachromosomal DNA and RNA. The term "isolated" thus encompasses nucleic acids purified by standard nucleic acid-purification methods. The term also embraces nucleic acids prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids.

35   DNA constructs incorporating an OsMADS1 gene r fragment there f according t the present invention preferably place the OsMADS1 pr tein coding s quence under the control f an operably linked promoter that is capable of expression in a plant cell. Various promoters suitable for expression f heterologous

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genes in plant cells are known in the art, including constitutiv promoters, e.g. the cauliflower mosaic virus (CaMV) 35S promoter, which is expressed in many plant tissues, organ- or tissue-specific promoters, and promoters that are inducible by chemicals such as methyl jasminate, salicylic acid, or Safener, for example.

5   Plant transformation and regeneration. In addition to the methods for plant transformation and regeneration described in the Examples below for making transgenic plants, other well-known methods can be employed.

Fragments, probes, and primers. A fragment of an OsMADS1 nucleic acid according to the present invention is a portion of the nucleic acid that is less than

10   full-length and comprises at least a minimum length capable of hybridizing specifically with an OsMADS1 nucleic acid according to the present invention (or a sequence complementary thereto) under stringent conditions as defined below. The length of such a fragment is preferably 15 nucleotides or more, although a shorter nucleic acid can be employed as a probe or primer if it is shown to specifically hybridize under

15   stringent conditions with a target nucleic acid by methods well known in the art.

Nucleic acid probes and primers can be prepared based on nucleic acids according to the present invention, e.g., the OsMADS1 gene of Fig. 1A or the NtMADS3 gene of Fig. 10. A "probe" comprises an isolated nucleic acid attached to a detectable label or reporter molecule well known in the art. Typical labels include

20   radioactive isotopes, ligands, chemiluminescent agents, and enzymes.

"Primers" are short nucleic acids, preferably DNA oligonucleotides 15 nucleotides or more in length, which are annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, then extended along the target DNA strand by a polymerase, preferably a

25   DNA polymerase. Primer pairs can be used for amplification of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods well known in the art. PCR-primer pairs can be derived from the sequence of a nucleic acid according to the present invention, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, © 1991,

30   Whitehead Institute for Biomedical Research, Cambridge, MA).

Methods for preparing and using probes and primers are described, for example, in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; Current Protocols in Molecular Biology, ed. Ausubel et al., Greene

35   Publishing and Wiley-Interscience, N w York, 1987 (with peri dic updates); and Innis et al., PCR Protocols: A Guide to Methods and Applications, Academic Press: San Diego, 1990.

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Pr bes r primers can be free in s lution r c valently or n nc valently attached to a solid supp rt by standard m ans.

Substantial similarity. A first nucleic acid is "substantially similar" to a second nucleic acid if, when optimally aligned (with appropriate nucleotide insertions

5   or deletions) with the other nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 75%-90% of the nucleotide bases, and preferably greater than 90% of the nucleotide bases. ("Substantial sequence complementarity" requires a similar degree of sequence complementarity.) Sequence similarity can be determined by comparing the nucleotide sequences of two nucleic

10 acids using sequence analysis software such as the Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, Madison, WI.

Alternatively, two nucleic acids are substantially similar if they hybridize under stringent conditions, as defined below.

15 Operably linked. A first nucleic-acid sequence is "operably" linked with a second nucleic-acid sequence when the first nucleic-acid sequence is placed in a functional relationship with the second nucleic-acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA

20 sequences are contiguous and, where necessary to join two protein coding regions, in reading frame.

"Recombinant". A "recombinant" nucleic acid is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial

25 combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques.

Techniques for nucleic-acid manipulation are described generally in, for example, Sambrook et al. (1989) and Ausubel et al. (1987, with periodic updates).

30 Preparation of recombinant or chemically synthesized nucleic acids; vectors, transformation, host cells. Large amounts of a nucleic acid according to the present invention can be produced by recombinant means well known in the art or by chemical synthesis.

Natural or synthetic nucleic acids according to the present invention can

35 be incorporated into rec mbinant nucl ic-acid constructs, typically DNA constructs, capable of introduction into and replication in a host cell. Usually the DNA constructs will be suitable for replication in a unic llular host, such as *E. coli* or other commonly

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used bacteria, but can also be introduced int yeast, mammalian, plant or ther eukaryotic cells.

Preferably, such a nucleic-acid construct is a vector comprising a replication system recognized by the host. For the practice of the present invention, well-known compositions and techniques for preparing and using vectors, host cells, introduction of vectors into host cells, etc. are employed, as discussed, *inter alia*, in Sambrook et al., 1989, or Ausubel et al., 1987.

A cell, tissue, organ, or organism into which has been introduced a foreign nucleic acid, such as a recombinant vector, is considered "transformed" or "transgenic." The DNA construct comprising an OsMADS1 DNA sequence according to the present invention that is present in a transgenic host cell, particularly a transgenic plant, is referred to as a "transgene." The term "transgenic" or "transformed" when referring to a cell or organism, also includes (1) progeny of the cell or organism and (2) plants produced from a breeding program employing such a "transgenic" plant as a parent in a cross and exhibiting an altered phenotype resulting from the presence of the recombinant OsMADS1 DNA construct.

Methods for chemical synthesis of nucleic acids are discussed, for example, in Beaucage and Carruthers, Tetra. Letts. 22:1859-1862, 1981, and Matteucci et al., J. Am. Chem. Soc. 103:3185, 1981. Chemical synthesis of nucleic acids can be performed, for example, on commercial automated oligonucleotide synthesizers.

Nucleic-Acid Hybridization; "Stringent Conditions"; "Specific". The nucleic-acid probes and primers of the present invention hybridize under stringent conditions to a target DNA sequence, e.g., to the rice OsMADS1 gene and its homologs in other plant species.

The term "stringent conditions" is functionally defined with regard to the hybridization of a nucleic-acid probe to a target nucleic acid (i.e., to a particular nucleic-acid sequence of interest) by the hybridization procedure discussed in Sambrook et al., 1989 at 9.52-9.55. *See also*, Sambrook et al., 1989 at 9.47-9.52, 9.56-9.58; Kanehisa, Nuc. Acids Res. 12:203-213, 1984; and Wetmur and Davidson, J. Mol. Biol. 31:349-370, 1968.

Regarding the amplification of a target nucleic- acid sequence (e.g., by PCR) using a particular amplification primer pair, stringent conditions are conditions that permit the primer pair to hybridize only to the target nucleic-acid sequence to which a primer having the corresponding wild-type sequence (or its complement) w uld bind.

Nucleic-acid hybridization is affected by such c nditi ns as salt concentration, temperature, r organic solvents, in addition to the base c mp sition,

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length of the c mplementary strands, and the number f nucleotid - base mismatches between the hybridizing nucleic acids, as will be readily appreciat d by those skilled in the art.

When referring to a probe or primer, the term "specific for (a target sequence)" indicates that the probe or primer hybridizes under stringent conditions only to the target sequence in a given sample comprising the target sequence.

Nucleic-acid amplification. As used herein, "amplified DNA" refers to the product of nucleic-acid amplification of a target nucleic-acid sequence. Nucleic-acid amplification can be accomplished by any of the various nucleic-acid amplification methods known in the art, including the polymerase chain reaction (PCR). A variety of amplification methods are known in the art and are described, inter alia, in U.S. Patent Nos. 4,683,195 and 4,683,202 and in PCR Protocols: A Guide to Methods and Applications, Innis et al. eds., Academic Press, San Diego, 1990.

In situ hybridization. A number of techniques have been developed in which nucleic-acid probes are used to locate specific DNA sequences on intact chromosomes in situ, a procedure called "in situ hybridization." See, e.g., Pinkel et al., Proc. Natl. Acad. Sci. USA 85:9138-9142, 1988 (regarding fluorescence in situ hybridization), and Lengauer et al., Hum. Mol. Genet. 2:505-512, 1993 (regarding "chromosomal bar codes"). Well-known methods for in situ hybridization and for the preparation of probes or primers for such methods are employed in the practice of the present invention, including direct and indirect in situ hybridization methods.

Methods of making cDNA clones encoding OsMADS1 or homologs thereof. Based upon the availability of the OsMADS1 and NtMADS3 cDNAs and the nucleotide sequences thereof, as disclosed herein, other OsMADS1 genes (e.g., alleles and homologs of OsMADS1 and NtMADS3) can be readily obtained from a wide variety of plants by cloning methods known in the art.

For example, one or more primer pairs based on the OsMADS1 sequence can be used to amplify such OsMADS1 genes or their homologs by the polymerase chain reaction (PCR). Alternatively, the disclosed OsMADS1 cDNA or fragments thereof can be used to probe a cDNA or genomic library made from a given plant species.

Cloning of the OsMADS1 Genomic Gene and Homologs Thereof. The availability of the OsMADS1 cDNA sequence enables those skilled in the art to obtain a genomic clone corresponding to the OsMADS1 cDNA (including the promoter and other regulatory regions and intron sequences) and the determination of its nucleotide sequence by conventional methods. Such an OsMADS1 genomic clone and sequences derived therefrom are useful, for example, for studies of the expression of the OsMADS1 gene.

Nucleotide-Sequence Variants of OsMADS1 cDNA and Amino Acid
Sequence Variants of OsMADS1 Protein. Using the nucl   tide and the amino-acid
sequence of the OsMADS1 protein disclosed herein, those skilled in the art can create
DNA molecules and polypeptides that have minor variations in their nucleotide or
5   amino acid sequence.

"Variant" DNA molecules are DNA molecules containing minor changes
in the native OsMADS1 sequence, i.e., changes in which one or more nucleotides of a
native OsMADS1 sequence is deleted, added, and/or substituted while substantially
maintaining OsMADS1 biological activity. Variant DNA molecules can be produced,
10   for example, by standard DNA mutagenesis techniques or by chemically synthesizing
the variant DNA molecule. Such variants do not change the reading frame of the
protein-coding region of the nucleic acid and encode a protein having no or only minor
changes in OsMADS1 biological function. Preferably, the mutations made in the
native OsMADS1 sequence do not create complementary regions that could produce
15   secondary mRNA structure (see, e.g., EP 75,444A).

Amino-acid substitutions are preferably substitutions of single amino-acid
residues. DNA insertions are preferably of about 1 to 10 contiguous nucleotides and
deletions are preferably of about 1 to 30 contiguous nucleotides. Insertions and
deletions are preferably insertions or deletions from an end of the protein-coding or
20   non-coding sequence and are preferably made in adjacent base pairs. Substitutions,
deletions, insertions or any combination thereof can be combined to arrive at a final
construct.

Preferably, variant nucleic acids according to the present invention are
"silent" or "conservative" variants. "Silent" variants are variants of a native rice
25   OsMADS1 sequence or a homolog thereof in which there has been a substitution of
one or more base pairs but no change in the amino-acid sequence of the polypeptide
encoded by the sequence. "Conservative" variants are variants of the native rice
OsMADS1 sequence or a homolog thereof in which at least one codon in the protein-
coding region of the gene has been changed, resulting in a conservative change in one
30   or more amino acid residues of the polypeptide encoded by the nucleic-acid sequence,
i.e., an amino acid substitution. A nu: ar of conservative amino acid substitutions
are listed in Table 3. In addition, one c: more codons encoding cysteine residues can
be substituted for, resulting in a loss of a cysteine residue and affecting disulfide
linkages in the OsMADS1 polypeptide.

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TABLE 1

Original Residue	Conservative Substitutions
Ala	ser
Arg	lys
Asn	gln, his
Asp	glu
Cys	ser
Gln	asn
Glu	asp
Gly	pro
His	asn; gln
Ile	leu, val
Leu	ile; val
Lys	arg; gln; glu
Met	leu; ile
Phe	met; leu; tyr
Ser	thr
Thr	ser
Trp	tyr
Tyr	trp; phe
Val	ile; leu

Substantial changes in function are made by selecting substitutions that are less conservative than those in Table 1, i.e., selecting residues that differ more significantly in their effect on maintaining: (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation; (b) the charge or hydrophobicity of the molecule at the target site; or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in protein properties are those in which: (a) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histadyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine.

Polypeptides

The term "OsMADS1 protein (or polypeptide)" refers to a protein encoded by an OsMADS1 gene, including alleles and homologs of OsMADS1, or by a variant of the OsMADS1 g ne, having OsMADS1 biological activity. An OsMADS1 polypeptide can be isolated from a natural source, produced by the expression of a recombinant OsMADS1 nucleic acid, or be chemically synthesized. Techniques for chemical

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synthesis of polypeptides are d scribed, f r example, in Merrifield, J. Amer. Chem. Soc. 85:2149-2156, 1963.

Polypeptide sequence homology. Ordinarily, the polypeptides encompassed by the present invention are at least about 70% homologous to a native

5   OsMADS1 polypeptide, preferably at least about 80% homologous, and more preferably at least about 95% homologous. Such homology is considered to be "substantial homology," although more important than shared amino-acid sequence homology can be the common possession of characteristic structural features and the retention of characteristic biological activity.

10   Polypeptide homology is typically analyzed using sequence analysis software such as the Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, Madison, WI). Polypeptide sequence analysis software matches homologous sequences using measures of homology assigned to various substitutions, deletions, substitutions, and other

15   modifications.

"Isolated," "Purified," "Homogeneous" Polypeptides. A polypeptide is "isolated" if it has been separated from the cellular components (nucleic acids, lipids, carbohydrates, and other polypeptides) that naturally accompany it. Such a polypeptide can also be referred to as "pure" or "homogeneous" or "substantially"

20   pure or homogeneous. Thus, a polypeptide which is chemically synthesized or recombinant (i.e., the product of the expression of a recombinant nucleic acid, even if expressed in a homologous cell type) is considered to be isolated. A monomeric polypeptide is isolated when at least 60-90% by weight of a sample is composed of the polypeptide, preferably 95% or more, and more preferably more than 99%.

25   Protein purity or homogeneity is indicated, for example, by polyacrylamide gel electrophoresis of a protein sample, followed by visualization of a single polypeptide band upon staining the polyacrylamide gel; high pressure liquid chromatography; or other methods known in the art.

Protein purification. The polypeptides of the present invention can be

30   purified by any of the means known in the art. Various methods of protein purification are described, e.g., in Guide to Protein Purification, ed. Deutscher, Meth. Enzymol. 185, Academic Press, San Diego, 1990; and Scopes, Protein Purification: Principles and Practice, Springer Verlag, New York, 1982.

Variant forms of OsMADS1 polypeptides; labeling. Encompassed by the

35   claimed OsMADS1 polypeptides are variant polypeptides in which there have been substitutions, deletions, insertions or other modifications of the native OsMADS1 polypeptide. The variants substantially retain structural and/or biological

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characteristics and are preferably silent or conservative substitutions f one or a small number of contiguous amino acid residues.

The native OsMADS1 polypeptide sequence can be modified by conventional methods, e.g., by acetylation, carboxylation, phosphorylation,
5   glycosylation, ubiquitination, and labeling, whether accomplished by *in vivo* or *in vitro* enzymatic treatment of an OsMADS1 polypeptide or by the synthesis of an OsMADS1 polypeptide using modified amino acids.

There are a variety of conventional methods and reagents for labeling polypeptides and fragments thereof. Typical labels include radioactive isotopes,
10   ligands or ligand receptors, fluorophores, chemiluminescent agents, and enzymes. Methods for labeling and guidance in the choice of labels appropriate for various purposes are discussed, e.g., in Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1989; and Current Protocols in Molecular Biology, ed. Ausubel et al., Greene
15   Publishing and Wiley-Interscience: New York, 1987 (with periodic updates).

Polypeptide Fragments. The present invention also encompasses fragments of OsMADS1 polypeptides that lack at least one residue of a native full-length OsMADS1 polypeptide yet retain at least one of the biological activities characteristic of rice OsMADS1. For example, the fragment can cause early flowering
20   or dwarf phenotypes when expressed as a transgene in a host plant or possession of a characteristic immunological determinant. As an additional example, an immunologically active fragment of an OsMADS1 polypeptide is capable of raising OsMADS1-specific antibodies in a target immune system (e.g., murine or rabbit) or of competing with OsMADS1 for binding to OsMADS1-specific antibodies, and is thus
25   useful in immunoassays for the presence of OsMADS1 polypeptides in a biological sample. Such immunologically active fragments typically have a minimum size of 7 to 17 amino acids.

Fusion polypeptides. The present invention also provides fusion polypeptides including, for example, heterologous fusion polypeptides, i.e., an
30   OsMADS1 polypeptide sequence or fragment thereof and a heterologous polypeptide sequence, e.g., a sequence from a different polypeptide. Such heterologous fusion polypeptides thus exhibit biological properties (such as ligand-binding, catalysis, secretion signals, antigenic determinants, etc.) derived from each of the fused sequences. Fusion partners include, f r example, immunoglobulins, beta
35   galactosidase, trpE, protein A, beta lactamase, alpha amylase, alcohol dehydrogenase, yeast alpha mating factor, and various signal and leader sequences which, e.g., can direct the secretion f the polypeptide. Fusion polypeptides are typically made by the expression of recombinant nucleic acids or by chemical synthesis.

Polypeptide sequence determination. The sequence of a polypeptide f the present inventi n can be determined by various methods kn wn in the art. In order to determine the sequence of a polypeptide, the polypeptide is typically fragmented, the fragments separated, and the sequence of each fragment determined.

5 To obtain fragments of an OsMADS1 polypeptide, the polypeptide can be digested with an enzyme such as trypsin, clostripain, or *Staphylococcus* protease, or with chemical agents such as cyanogen bromide, o-iodosobenzoate, hydroxylamine or 2-nitro-5-thiocyanobenzoate. Peptide fragments can be separated, e.g., by reversed-phase high-performance liquid chromatography

10 (HPLC) and analyzed by gas-phase sequencing.

Polypeptide coupling to a solid phase support. The polypeptides of the present invention can be free in solution or coupled to a solid-phase support, e.g., nitrocellulose, nylon, column packing materials (e.g., Sepharose beads), magnetic beads, or glass wool.

15 Antibodies

The present invention also encompasses polyclonal and/or monoclonal antibodies capable of specifically binding to OsMADS1 polypeptides and fragments thereof, produced by conventional methods. Such antibodies are raised against an OsMADS1 polypeptide and are capable of distinguishing the OsMADS1 polypeptide

20 from other polypeptides.

An immunological response is usually assayed with an immunoassay. Normally such immunoassays involve some purification of a source of antigen, for example, produced by the same cells and in the same fashion as the antigen was produced.

25 For the preparation and use of antibodies according to the present invention, including various immunoassay techniques and applications, see, e.g., Goding, Monoclonal Antibodies: Principles and Practice, 2d ed, Academic Press, New York, 1986; and Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988. OsMADS1-specific antibodies are

30 useful, for example in purifying OsMADS1 polypeptides from a biological sample, such as a host cell expressing recombinant OsMADS1 polypeptides; in cloning OsMADS1 homologs from rice or other plant species from an expression library; as antibody probes for protein blots and immunoassays; etc.

Frequently, the polypeptides and antibodies will be labeled by joining,

35 either covalently or noncovalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known. Suitable labels include radi nuclides, enzymes, substrates, cofactors, inhibitors, fluorescent agents, chemiluminescent agents, magnetic particles and the like.

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The invention will be better underst   d by reference t   the following Examples, which are intended t   merely illustrate the best mode n w kn wn f r practicing the invention. The scope of the invention is not to be considered limited thereto, however.

5   EXAMPLES

EXAMPLE 1: Isolation and Analysis of a MADS-Box Gene from Rice, OsMADS1

Bacterial Strains, Plant Materials, and Plant Transformation. *Escherichia coli* MC1000 (ara, leu, lac, gal, str) was used as the recipient for routine cloning experiments. Rice (*Oryza sativa L.* cv. M201) plants were grown in a growth chamber
10   at 26°C with 10.5-hr day cycle.

cDNA Library Construction and Molecular Characterization. A cDNA library was constructed using the λZapII vector (Stratagene, La Jolla, CA) and poly(A)+ mRNA isolated from rice flowers. An adapter containing *EcoRI* and *NotI* sites (Pharmacia LKB Biotechnology, Piscataway, NJ) was used to ligate the vector
15   and cDNA. The library was divided into 20 sublibraries and amplified in an *E. coli* host strain, XL-1 Blue [F'::Tn10 pro+B+, laclq, (lac Z)MI5/recAJ. endAI, gyrA96 (Nar), thi, hsdR17(rk⁻, mk⁺), sup44, reLA1, lac] (Stratagene, La Jolla, CA).

Plaque hybridization was performed with 10^6 plaques that were lifted onto nitrocellulose membranes. The plasmid pBluescript containing the OsMADS1 cDNA
20   was rescued *in vivo* from the bacteriophage λ using fl helper phage, R408 (Stratagene, La Jolla, CA). Both strands of the cDNA inserts were sequenced by the dideoxynucleotide chain-termination method using double-stranded DNA as a template (Sanger et al., Proc. Natl. Acad. Sci. USA 74:5463-5467, 1977).

Southern and Northern Blot Analyses. Genomic DNA was prepared from
25   two-week-old rice seedlings by the CTAB (cetyltrimethylammonium bromide) method (Rogers and Bendich, Extraction of DNA from plant tissues, In: Gelvin and Schilperoort, eds., Plant Molecular Biology Manual, Kluwer Academic, Dordrecht, Belgium, 1988, pp. A6/1-10). Four μg of DNA were digested with appropriate restriction enzymes, separated on a 0.7% agarose gel, blotted onto a nylon
30   membrane, and hybridized with a ^{32}P-labeled probe labeled by the random-priming method (Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989). Ten μg of total RNA isolated by the guanidium thiocyanate method were used for the northern analysis (*id.*).

35   In situ Localization. Rice flowers were dehydrated with ethanol, fixed (1.4% glutaraldehyde, 2% paraformaldehyde, 50 mM PIPES, pH 7.2), and embedded in paraffin. Eight-μm sections were attached to gelatin-coated glass slides and hybridized with ^{35}S-labeled antisense RNA (Cox and Goldberg, Analysis of plant gene

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xpression, In: Shaw, ed., Plant M lecular Bi logy: A Practical Approach, IRL Press, Oxford, 1988, pp. 1-34). Th RNA pr be was pr pared by *in vitro* transcription using pBluescript carrying the OsMADS1 cDNA clone as a template. The sections were coated with an X-ray emulsion film and exposed for four days. The samples were

5   stained with 0.5% toluidine blue to visualize tissue sections. Photographs were taken with a bright-field microscope.

Results. A cDNA clone, OsMADS1, was isolated by screening a *λ* ZapII cDNA library prepared from immature rice flower mRNA using mixed probes of different MADS-box cDNA clones isolated from *Arabidopsis* (Ma et al., Genes Dev.

10   5:484-495, 1991; Yanofsky et al., Nature 346:35-39, 1990), *Brassica* (Mandel et al., Cell 71:133-143, 1992), tobacco (Kempin et al., Plant Physiol. 103:1041-1046, 1933), and tomato (Pnueli et al., Plant J. 1:255-266, 1991).

DNA sequence analysis showed that the rice clone encodes a protein of 257 amino-acid residues (Fig. 1A). The deduced amino-acid sequence contains the

15   conserved MADS-box domain between amino acids 2 and 57 (Fig. 1B). A second domain present in MADS-box proteins, the "K-box," is located between residues 90 and 143. The OsMADS1 clone appears to be nearly full length, since the estimated transcript length by northern hybridization analysis is similar to that of the cDNA clone. The conserved MADS-box region is located immediately after the start methionine

20   codon in the rice gene, as has been observed in most MADS-box genes. Therefore it is unlikely that the rice clone encodes a truncated protein.

These observations indicate that OsMADS1 is a member of the MADS-box gene family. Among characterized MADS-box proteins, the OsMADS1 amino-acid sequence is most homologous to *AP1* (44.4% identity) and *SQUA* (42.6% identity).

25   In addition, OsMADS1 shows extensive similarity to the functionally anonymous *Arabidopsis* MADS-box genes *AGL2* (56.2% identity) and *AGL4* (55.4% identity).

To determine the number of MADS-box genes present in rice, Southern blot analysis was performed. Figs. 2A and 2B show Southern blots of rice DNA digested with *EcoRI* (E), *HindIII* (H), or *PstI* (P), fractionated on a 0.7% agarose gel,

30   and hybridized with a probe prepared from the entire OsMADS1 cDNA (Fig. 2A) or an OsMADS1 cDNA probe lacking the conserved MADS-box region (Fig. 2B). More than ten restriction fragments hybridized with the entire cDNA probe, whereas a single fragment was detected by a probe lacking the conserved MADS-box region. This result indicates that the rice genome contains a large number of genes encoding

35   MADS-box proteins, similar to what is observed in other plant species (Angenent et al., Plant Cell 4:983-993, 1992; Ma, Genes Dev. 5:484-495, 1991; Pnueli et al., Plant J. 1:255-266, 1991; Schmidt et al., Plant Cell 5:729-737, 1993).

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    N rthern blot analyses were conducted t study the expressi n pattern of
the OsMADS1 gene in rice. Figs. 3A and 3B show the OsMADS1 expression pattern
in rice organs. Ten-μg samples of total RNA isolated from leaf (L) and root (R) of two-
week-old seedlings, and anther (A), carpel (C), and palea/lemma (P) of anthesis-stage

5 flowers was hybridized with the OsMADS1 probe lacking the MADS domain. Ethidium
bromide staining of 25S and 18S rRNAs demonstrated equal amounts of RNA loading.

    Figs. 3A and 3B also show the temporal expression pattern during flower
development. Twenty μg of total RNA was isolated from rice flowers at different
developmental stages: young inflorescence (panicle size < 1 cm), young flowers

10 (panicle size = 1 to 6 cm), flowers at the early vacuolated pollen stage, and flowers
at the late vacuolated pollen stage. This RNA was used for detection of OsMADS1
gene expression. RNA samples were hybridized with the probe lacking the conserved
MADS-box region in order to avoid cross-hybridization with other MADS-box genes
and thus to observe the specific expression pattern of the gene. OsMADS1

15 transcripts were present in the palea, lemma, and carpel, but not in the anther or
vegetative organs (Fig. 3A). The gene was active during the young inflorescence
stage and expression continued into the early and late vacuolated pollen stages (Fig.
3B).

    Figs. 4A-4C show the localization of the OsMADS1 transcript in rice

20 flowers and phenotypes of transgenic tobacco plants expressing OsMADS1 by *in situ*
hybridization experiments using longitudinal sections of young inflorescence, and cross
sections of the upper and lower rice flower at the vacuolated pollen stage (anther,
filament, flower primordia, lemma, ovary, palea, sheath, and sterile lemma). 8-μm
sections were hybridized with [36]S-labeled antisense RNA lacking the MADS-box

25 domain. The sections were coated with an X-ray emulsion film and exposed for four
days. The samples were stained with 0.5% toluidine blue to visualize tissue sections
which show negative expression of the gene. A sense probe did not show any
hybridization above the background level. These *in situ* experiments revealed that the
OsMADS1 transcript was uniformly present in young flower primordia during early

30 flower development and later became localized in certain floral organs (Figs. 4A-4C).
In young inflorescences, strong hybridization signals were detected in flower primordia
but not in other tissues (Fig. 4A). In vacuolated pollen-stage flowers, OsMADS1
mRNA was detected in the palea, lemma, and ovary. However, the hybridization
signal was not uniform in these tissues. In particular, the tissues near the

35 palea/lemma junction and the palea tissues covered by lemma exhibited little or no
expression of the gene. No significant signal was observed in the anther, filament, or
sterile lemma. These results indicate that the OsMADS1 gene is preferentially
expressed in certain floral tissues, as has been observed with most MADS-box genes.

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The xpressi n patt rn f the OsMADS1 g ne cl sely resembled that of *AP1* and *SQUA* (Juijser et al., EMBO J. 11:1239-1249, 1992; Mandel t al., Nature 360:273-277, 1992). Flower-specific expression is also common for other MADS-box genes (Angenant et al., Plant Cell 4:983-993, 1992; Jack et al., Cell 68:683-697, 1992; Kempin et al., Plant Physiol. 103:1041-1046, 1993; Ma et al., Genes Dev. 5:484-495, 1991; Mandel et al., Nature 360:273-277, 1992; Pnueli et al., Plant J. 1:255-266, 1991; Schmidt et al., Plant Cell 5:729-737, 1993; Sommer et al., EMBO J. 9:605-613, 1990; Tsuchimoto et al., Plant Cell 5:843-853, 1993).

Nine independent clones that contain the conserved MADS-box have been isolated.

EXAMPLE 2: Expression of OsMADS1 in Transgenic Tobacco Plants Results in Early Flowering and Dwarf Phenotypes

Bacterial Strains, Plant Materials, and Plant Transformation.

Agrobacterium tumefaciens LBA4404 (Hoekema et al., Nature 303:179-181, 1983), containing the Ach5 chromosomal background and a disarmed helper-Ti plasmid pAL4404, was used for transformation of tobacco plants (*Nicotiana tabacum* L. cv. Petit Havana SR1) by the co-cultivation method (An et al., Binary Vectors, In: Gelvin and Schilperoort, eds., Plant Molecular Biology Manual, Kluwer Academic, Dordrecht, Belgium, 1988, pp. A3/1-19). Transgenic plants were maintained in a greenhouse.

Results. Ectopic expression of floral homeotic genes alters floral organ identity in homologous (Kempin et al., Plant Physiol. 103:1041-1046, 1993; Mizukami and Ma, Cell 71:119-131, 1992; Pnueli et al., Plant Cell 6:163-173, 1994; Tsuchimoto et al., Plant Cell 5:843-853, 1993) and heterologous systems (Mandel et al., Cell 71:133-143, 1992).

In order to characterize the functional role of OsMADS1, tobacco plants were used as a heterologous expression system. The cDNA clone encoding the entire OsMADS1 coding region was placed under the control of cauliflower mosaic virus 35S promoter (Benfey and Chua, Science 250:959-966, 1990) and transcript 7 terminator using a binary vector pGA748, which is a derivative of pGA643 (An et al., Binary Vectors, In: Gelvin and Schilperoort, eds., Plant Molecular Biology Manual, Kluwer Aca emic, Dordrecht, Belgium, 1988, pp. A3/1-19). The chimeric molecule (p 209) was transferred to tobacco (*Nicotiana tabacum* cv. Petite Havana SR1) plar s using the *Agrobacterium*-mediated Ti plasmid vector system (An et al., Binary Vectors, In: Gelvin and Schilperoort, eds., Plant Molecular Biology Manual, Kluwer Academic, Dordrecht, Belgium, 1988, pp. A3/1-19). Twenty independ nt transgenic plants were studied.

Most of the primary transgenic plants flowered much earlier than control plants that were transformed with the Ti plasmid vect r alone. The transgenic plants

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were significantly shorter and contained several lateral branches (Figs. 4D-4E). Thes
phen types were inherited t the next generation as a dominant Mendelian trait.

Northern-blot analysis was conducted on seven transgenic plants which
displayed the early flowering phenotype (Fig. 5). Transcripts from a control plant (C)

5   and seven different transgenic plants (1-7) exhibiting the early flowering and dwarf
phenotypes were sampled for preparation of total RNA from leaves and flowers.
Twenty μg of total RNA was hybridized with
^{32}P-labeled probe prepared from the OsMADS1 cDNA lacking the MADS domain. The
results (Fig. 5) showed that all of the transgenic plants accumulated the OsMADS1

10 transcripts in both vegetative and reproductive organs. Although there were
significant differences in gene expression among the transgenic plants, the relative
expression level was similar between the leaf and flower. Transgenic plant #7, which
displayed the most severe symptoms, accumulated the highest level of the transcript.
Plants #4, #5, #6, with less severely altered phenotypes, expressed the gene at

15 reduced levels, indicating that the level of OsMADS1 RNA correlated with phenotype.

However, progeny from the same parent displayed phenotypic variation.
The basis of this variation was investigated with T1 offspring of the transgenic plant
#2 in which the transgene segregates as a single locus. OsMADS1 homozygotes were
much shorter (34.2 ± 0.8 cm) compared to heterozygotes (51.6 ± 1.4 cm), while the

20 wild-type tobacco plants were 119.8 ± 2.2 cm. The homozygotes flowered two days
earlier than the heterozygotes and eight days earlier than wild type plants. This result
indicates that the variation was due to gene dosage.

Table 2 summarizes characteristics of four independently transformed
plants from the T1 generation. Seeds were collected from selfed fruits of the primary

25 transgenic plants (T0 generation). The seeds were germinated in a peat pellet and
grown for two weeks at 16 hr light/8 hr dark cycles under fluorescent lights. The
resulting T1 plants were grown under greenhouse conditions. Ten to twenty plants
were analyzed for each transgenic line. Standard errors are shown in parentheses.
Progeny carrying the transgenes were identified by visually scoring T2 seedlings for

30 kanamycin resistance. The kanamycin-sensitive segregants were used as controls (C).
Days to flowering include the time from seed germination to the first anthesis. Height
and internode length were measured when fruits were fully developed (90 days
postgermination). The data in Table 2 show that transgenic plants flowered 7 to 10
days earlier than wild-type plants and their height and internode length appear to be

35 significantly reduced.

TABLE 2:   Comparison of phenotypes of transgenic plants with non-transformed control

Transgenic Line (#)	Days to Flowering	Height (cm)	Internode Length (cm)
1	53.0 (2.0)	61.2 (5.8)	5.7 (0.5)
2	54.2 (0.3)	47.6 (1.9)	4.6 (0.2)
3	53.0 (0.4)	64.3 (3.5)	5.8 (0.3)
4	50.6 (0.9)	40.2 (4.4)	3.5 (0.3)
C	61.0 (0.2)	119.8 (2.2)	9.0 (0.3)

EXAMPLE 3: Ectopic Expression of OsMADS1 Overcomes Photoperiod Dependency of Long-Day and Short-Day Flowering Plants

Transgenic plants that constitutively express a rice MADS-box gene, OsMADS1, flower earlier than untransformed controls, indicating that the OsMADS1 gene is involved in controlling flowering time.

Nicotiana sylvestris, a long-day flowering plant, and N. tabacum cv. Maryland Mammoth, a short-day flowering plant, were transformed with pGA1209, which contains a kanamycin selectable marker and a chimeric fusion between the CaMV 35S promoter and OsMADS1-coding region by the Agrobacterium-mediated co-cultivation method (An et al., Binary vectors, In Gelven and Schilperoort, eds., Plant Molecular Biology Manual A3:1-19, Kluwer Academic Publishers, Dordrecht, Belgium, 1988). Transgenic plants were regenerated on kanamycin-containing culture medium. Transgenic plants were selfed and kanamycin-resistant T1 offspring were used for the entire experiment. Plants were grown under either a short-day growth condition (10 hr light) or a long-day growth condition (16 hr light).

Total RNA was isolated from leaves of transgenic plants by the guanidium thiocyanate method (Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, NY, 1989). Twenty μg of total RNA was electrophoresed on a 1.3% agarose gel, blotted onto a nylon membrane, and hybridized with a ^{32}P-labeled probe prepared by the random-priming method (Sambrook et al., Molecula Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, NY, 1E. .).

Transgenic N. sylvestris flowered earlier than untransformed controls under the permissive flowering (long-day) conditions. Plants were short and branched with clustered flowers compared to the controls. These phenotypes are similar to day-neutral transgenic tobacco plants expressing the OsMADS1 gene.

In order to confirm whether the phenotypes were stably inherited, five independently transformed transgenic plants were chosen for further studies. T1

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offspring were selected n a kanamycin-containing medium and the seedlings were grown under the long-day r short-day c nditions. Under the l ng-day conditi n, the transgenic plants flowered 7-11 days earlier than the controls which flowered in 106 days after seed germination, as shown in Fig. 6A. The data are summarized in Table 3.

TABLE 3:   Ectopic expression of OsMADS1 in _Nicotiana sylvestris_

Transgenic Plant	Short Day Condition (10h)		Long Day Condition (16h)	
	Days to Flowering	Height (cm)	Days to Flowering	Height (cm)
1	102	62	98	68
2	85	35	95	45
3	146	65	99	72
4	84	36	96	46
5	97	52	97	52
control	-	-	106	85

The transgenic plants also showed short and branched phenotypes. When the transgenic plants were grown under the short-day (non-permissive) condition, they flowered within 85-146 days, whereas the untransformed control plants did not flower (Fig. 6B, Table 3). Transgenic line 2 and 4 flowered earlier under the short-day condition and the line 3 flowered under the long-day condition, while the line 1 and 5 flowered at approximately the same time.

In order to confirm whether the phenotypes observed resulted from the expression of the OsMADS1 gene, RNA blot analysis was performed. Since a constitutive promoter was used for expression of the gene, it was expected that the transcript was present in all the plant parts, since the 35S promoter-driven OsMADS1 transcript is almost equally expressed in both leaves and flowers. Total RNA was prepared from fully expanded leaves of five transgenic lines and the level of OsMADS1 transcript in each line was measured using the OsMADS1 cDNA probe (Fig. 7).

All the transgenic plants expressed the OsMADS1 transcript. The amount of the transcript was in direct correlation with the degree of the phenotypes. Transgenic lines 2 and 4, which flowered earliest, expressed the highest level of the OsMADS1 mRNA, whereas line 3, which flowered latest among the five transgenic lines, expressed the lowest level of the OsMADS1 mRNA. Transgenic lines with interm diate phenotypes expressed intermediate levels of the transcript.

These results suggest that xpression of the OsMADS1 gene caused a change in the timing of fl wering in a long-day flowering plant _N. sylvestris_. Under

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permissive l ng-day conditions, transgenic plants fl wer d earlier than controls. Under n n-permissive sh rt-day c nditions, expr ssi n of the transgene overcame the day-length requirement for flowering. The degree of the phenotype correlated with the level of expression of the transgene, especially under short-day conditions.

5 Interestingly, transgenic plants expressing a high level of the OsMADS transcript flowered earlier under short-day conditions than under long-day conditions, the latter being permissive flowering conditions for untransformed *N. sylvestris*.

Expression of the OsMADS1 gene can also overcome the day-length requirement of a short-day flowering plant, *N. tabacum* cv. Maryland Mammoth.

10 Transgenic plants were obtained that expressed the OsMADS1 chimeric molecule. As observed with the day-neutral or long-day plant, transformation of the OsMADS1 chimeric gene into the short-day plant resulted in early flowering and bushy phenotypes under a short-day (permissive) condition.

Three independently transformed lines were further studied. T1 offspring

15 were selected on kanamycin-containing medium and grown under a short-day (permissive) or a long-day (non-permissive) conditions. Under permissive conditions, T1 transgenic lines flowered 16-21 days earlier than untransformed controls, which flowered in 119 days (Fig. 8A, Table 4). The height of the transgenic plants was less than one-half that of the control plants. Under non-permissive conditions, transgenic

20 plants flowered in 202-206 days, whereas the control did not flower (Fig. 8B, Table 4).

RNA blot analysis showed that all three lines expressed the OsMADS1 transcript (Fig. 9). Again, the degree of the phenotype correlated with the level of OsMADS1 transgene expression. Thus, expression of the OsMADS1 gene also

25 overcame the day-length requirement of a short-day plant.

TABLE 4: Ectopic expression of OsMADS1 in *Nicotiana tabacum* cv. Maryland Mammoth

Transgenic Plant	Short Day Condition (10h)		Long Day Condition (16h)	
	Days to Flowering	Height (cm)	Days to Flowering	Height (cm)
1	98	61	202	102
2	103	65	206	105
3	98	63	203	104
control	119	143	–	–

Ectopic expression of OsMADS1 overcomes the day-length dependence of flowering. The effect was more vident when the gene was highly expressed. The fact that OsMADS1 overcomes the day-length dependence of both short-day and long-

40 day plants indicates that a common gene product controls the timing of flowering in

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both short-day and long-day plants. It is likely that, under natural conditi ns,
expression of the OsMADS1 gene is tightly controlled by envir nmental c nditions and
the flowering process is initiated by triggering OsMADS1 gene expression.

EXAMPLE 4: Isolation and Analysis of a MADS-Box Gene from *Nicotiana tabacum*,
5   NtMADS3

A homolog of OsMADS1 was isolated from a *Nicotiana tabacum* cDNA
library constructed using the λZapII vector (Stratagene, La Jolla, CA) and poly(A) +
mRNA isolated from tobacco flowers as described above. Using the OsMADS1 cDNA
as a probe under moderately stringent hybridization conditions (60°C) in an initial
10   screen, several tobacco MADS-box genes were obtained.

In a secondary screen to identify a tobacco homolog of OsMADS1, the
OsMADS1 cDNA was split into two parts between the MADS-box and K-box
sequences and used to probe the tobacco MADS-box cDNAs. Only one of the cDNAs,
NtMADS3, hybridized to both halves of the OsMADS1 sequence, i.e., to the half
15   containing the MADS-box sequence and the half containing the OsMADS1 K-box
sequence.

To further confirm the identity of NtMADS3 as a homolog of OsMADS1,
all isolated tobacco MADS-box genes obtained in the primary screening of the tobacco
flower library were placed under the control of the 35S promoter and transformed into
20   *N. tabacum* as described above. Only transgenic *N. tabacum* expressing the
NtMADS3 transgene exhibited early flowering and dwarf phenotypes.

The nucleotide sequence of the NtMADS3 cDNA was obtained and
compared to OsMADS1. NtMADS3 is 945 bp long and contains an open reading
frame of 242 amino acid residues (Fig. 10). The deduced NtMADS1 polypeptide
25   sequence showed 56% homology with that of OsMADS1, with 96.5% homology in
the MADS-box and 77.3% homology in the K-box (Fig. 11).

All publications and published patent documents cited in this specification
are incorporated herein by reference to the same extent as if each individual
publication or patent application was specifically and individually indicated to be
30   incorporated by reference.

This invention has been detailed both by example and by direct .
description. It should be apparent that one having ordinary skill in the relevant art
would be able to surmise equivalents to the invention as described in the claims which
follow but which would be within the spirit of the foregoing description. Those
35   equivalents are to be included within the scope of this invention.

CLAIMS

1. An isolated nucleic acid c mprising at least 15 contigu us nucleic acids of a sequence selected from the group consisting of:

   (a) a native OsMADS1 gene; and

   (b) a variant OsMADS1 gene that is substantially similar in sequence to a native OsMADS1 gene and, when expressed in a transgenic plant, produces at least one phenotype in the transgenic plant selected from the group consisting of: (i) diminished apical dominance, (ii) early flowering, (iii) altered daylength requirement for flowering; (iv) greater synchronization of flowering; and (v) relaxed vernalization requirement.

2. The nucleic acid of claim 1 wherein the sequence is a native OsMADS1 gene.

3. The nucleic acid of claim 2 wherein the sequence is a native OsMADS1 gene from *Oriza sativa* or *Nicotiana tabacum*.

4. The nucleic acid of claim 3 wherein the sequence is that shown in Fig. 1A or Fig. 10.

5. The nucleic acid of claim 1 wherein the sequence is a variant OsMADS1 gene that comprises only silent or conservative substitutions to a native OsMADS1 gene sequence.

6. A vector comprising the nucleic acid of claim 1.

7. A host cell comprising the nucleic acid of claim 1.

8. A transgenic plant comprising the nucleic acid of claim 1.

9. A purified polypeptide encoded by the nucleic acid of claim 1.

10. A method of using the host cell of claim 7, the method comprising the steps of:

   providing the host cell; and

   culturing the host cell under conditions suitable for expression of the DNA sequence to produce an OsMADS1 polypeptide.

11. A method of producing an OsMADS1 polypeptide, the method comprising the steps of:

   providing the host cell of claim 7; and

   culturing the host cell under conditions suitable for expression of the DNA sequence to produce the OsMADS1 polypeptide.

12. A method of using a nucleic acid of claim 1 comprising the steps of:

   introducing the nucleic acid of claim 1 into a plant cell, thereby producing a transformed plant cell; and

   growing a plant having an altered phenotype from the transform d plant cell.

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13. A nucleic acid comprising a fragment f an OsMADS1 gene capable

f hybridizing under stringent conditi ns to a native OsMADS1 gene sequence.

14. The nucleic acid of claim 13 further comprising a detectable label.

15. A method of obtaining an allele or homolog of the OsMADS1 gene

5   from a plant species comprising the steps of:

providing a nucleic acid of the plant species;

contacting the nucleic acid of the plant species with a DNA molecule

under conditions allowing hybridization of the nucleic acid of the plant species and the

DNA molecule, thereby producing a hybridization product, wherein the DNA molecule

10   comprises a sequence from the group consisting of (i) a native OsMADS1 gene and (ii)

a fragment of a native OsMADS1 gene capable of hybridizing under stringent

conditions with the native OsMADS1 gene; and

identifying the allele or homolog.

16. The method of claim 15 wherein the DNA molecule comprises a

15   detectable label.

17. The method of claim 15 further comprising performing an

amplification reaction, thereby producing an amplification product, and the step of

identifying comprises observing the amplification product.

18. An allele or homolog of the OsMADS1 gene produced by the method

20   of claim 15.

19. A method of producing a plant having at least one altered phenotype

selected from the group consisting of (i) diminished apical dominance, (ii) early

flowering, (iii) altered daylength requirement for flowering, (iv) greater synchronization

of flowering, and (v) relaxed vernalization requirement, the method comprising the

25   steps of:

(a) introducing into a cell of the plant a nucleic acid of claim 1, thereby

producing a transformed cell;

(b) growing a plant having the altered phenotype from the transformed

cell.

30   20. A plant of a long-day or short-day species having a substantially

relaxed daylength requirement for flowering.

21. The plant of claim 20 having a completely relaxed daylength

requirement for flowering.

22. A method of producing multiple crops of flowers or fruits in a year

35   from the plant of claim 20, th method comprising the steps of:

(a) planting a reproductive unit of the plant;

(b) growing the planted reproductive unit under natural light conditions;

(c) harvesting flowers or fruits of the plant; and

(d) repeating steps (a) thr ugh (c) at least nce in the year.

23. A transgenic plant exhibiting a phenotype that is significantly different from nontransgenic control plants with respect to at least one phenotype selected from the group consisting of:

5   (a) early flowering;

(b) altered daylength requirement for flowering; (c) greater synchronization of flowering; and

(d) relaxed vernalization requirement.

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   1 AAAACTAGCTTGCAAAGGGGATAGAGTAGTAGAGAGAGAGAGAGAGGAGAGGAGGAGGAA

  61 GAAGATGGGGAGGGGGAAGGTGGAGCTGAAGCGGATCGAGAACAAGATCAGCCGGCAGGT           MADS
       MetGlyArgGlyLysValGluLeuLysArgIleGluAsnLysIleSerArgGlnVa   19     -box

 121 GACGTTCGCCAAGCGCAGGAACGGCCTGCTCAAGAAGGCCTACGAGCTCTCCCTCCTCTG
      lThrPheAlaLysArgArgAsnGlyLeuLeuLysLysAlaTyrGluLeuSerLeuLeuCy   39

 181 CGACGCCGAGGTCGCCCTCATCATCTTCTCCGGCCGCGGCCGCCTCTTCGAGTTCTCCAG
      sAspAlaGluValAlaLeuIleIlePheSerGlyArgGlyArgLeuPheGluPheSerSe   59

 241 CTCATCATGCATGTACAAAACCTTGGAGAGGTACCGCAGCTGCAACTACAACTCACAGGA
      rSerSerCysMetTyrLysThrLeuGluArgTyrArgSerCysAsnTyrAsnSerGlnAs   79

 301 TGCAGCAGCTCCAGAAAACGAAATTAATTACCAAGAATACCTGAAGCTGAAAACAAGAGT
      pAlaAlaAlaProGluAsnGluIleAsnTyrGlnGluTyrLeuLysLeuLysThrArgVa   99

 361 TGAATTTCTTCAAACCACACAGAGAAATATTCTTGGTGAGGATTTGGGCCCACTAAGCAT
      lGluPheLeuGlnThrThrGlnArgAsnIleLeuGlyGluAspLeuGlyProLeuSerMe   119    K-box

 421 GAAGGAGCTGGAGCAGCTTGAGAACCAGATAGAAGTATCCCTCAAACAAATCAGGTCAAG
      tLysGluLeuGluGlnLeuGluAsnGlnIleGluValSerLeuLysGlnIleArgSerAr   139

 481 AAAGAACCAAGCACTGCTTGATCAGCTGTTTGATCTGAAGAGCAAGGAGCAACAGCTGCA
      gLysAsnGlnAlaLeuLeuAspGlnLeuPheAspLeuLysSerLysGluGlnGlnLeuGl   159

 541 AGATCTCAACAAAGACTTGAGGAAAAAGTTACAGGAAACCAGTGCACAGAATGTGCTCCA
      nAspLeuAsnLysAspLeuArgLysLysLeuGlnGluThrSerAlaGluAsnValLeuHi   179

 601 TATGTCCTGGCAAGATGGTGGTGGGCACAGCGGTTCTAGCACTGTTCTTGCTGATCAGCC
      sMetSerTrpGlnAspGlyGlyGlyHisSerGlySerSerThrValLeuAlaAspGlnPr   199

 661 TCATCACCATCAGGGTCTTCTCCACCCTCACCCAGATCAGGGTGACCATTCCCTGCAGAT
      oHisHisHisGlnGlyLeuLeuHisProHisProAspGlnGlyAspHisSerLeuGlnIl   219

 721 TGGGTATCATCACCCTCATGCTCACCATCACCAGGCCTACATGGACCATCTGAGCAATGA
      eGlyTyrHisHisProHisAlaHisHisHisGlnAlaTyrMetAspHisLeuSerAsnGl   239

 781 AGCAGCAGACATGGTTGCTCATCACCCCAATGAACACATCCCATCCGGCTGGATATGATG
      uAlaAlaAspMetValAlaHisHisProAsnGluHisIleProSerGlyTrpIle***   257

 841 TGTGTGTTCAGTTCAGGCTTCAGGCTTCAGAGAAGCCAATGCAAACAGTGTCCTGTAATC

 901 CAGTAATTACAGGGCATATGTAATGTAATGTAATGTAATCCCTGATCTATATTTTGCTAA

 961 GTACGTGCGTGCTCTCTTACGACCTTCTCCCCCAAACAGTTAATCAGGGGAATAATAATT

1021 TCGTTTGATGCACGTACTGTATGTCTGTATCTGTCACTGTATCGTAGGACCGTCCATGTA

1081 TAACAATTTCCGTTTTGGATGTGGTAACAATTAATTGGCACTTAAATTTATATTTGTGAT

1141 G(A)n
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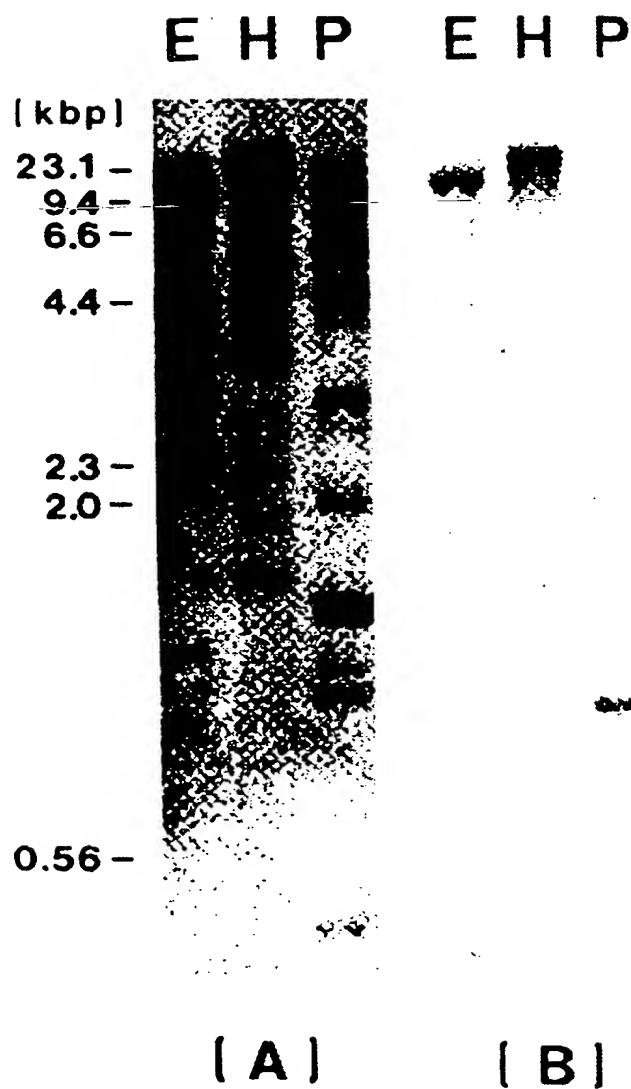
FIG. 1A

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GRGKVELKRIENKISRQVTFAKRRNGLLKKAYELSLLCDAEVALIIFSGRGRLFEF    OsMADS1

***R*Q*********N****S***A*******H*I*V**********VV**HK*K***Y    AP1
*****Q*********N****S***G*******H***V**********V**NK*K***Y    SQUA

****I*I*******TTN*****C********************V***********V**S****Y*Y    AG
****I*I*******ITN*****C********************V**********VV**S****Y*Y    PLE

*G*R*Q*******QTN****YS*******F***H**TV****R*SI*M**SSNK*H*Y    AP3
A***IQI******QTN****YS*******F***H***V****K*SI*MI*STQK*H*Y    DEF  A
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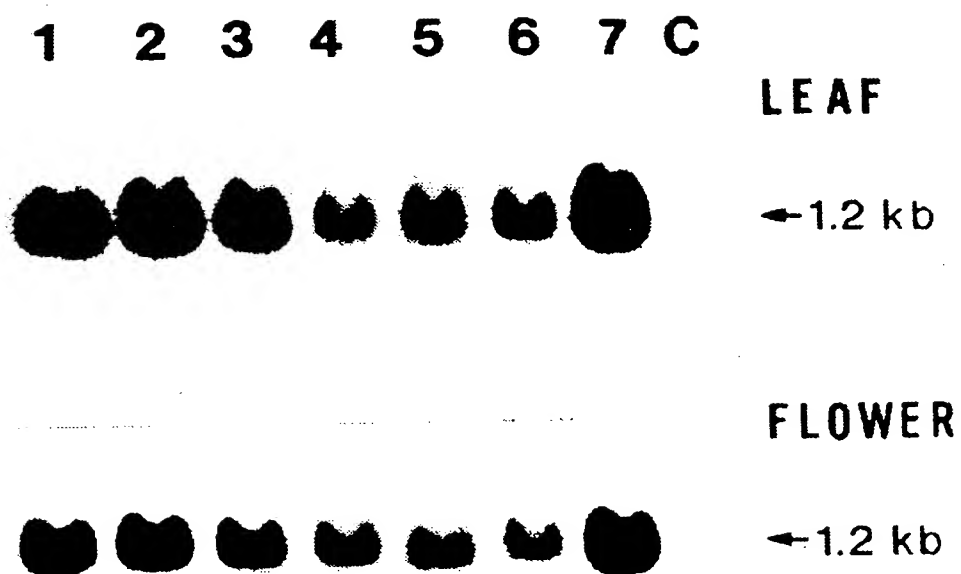
FIG. 1B
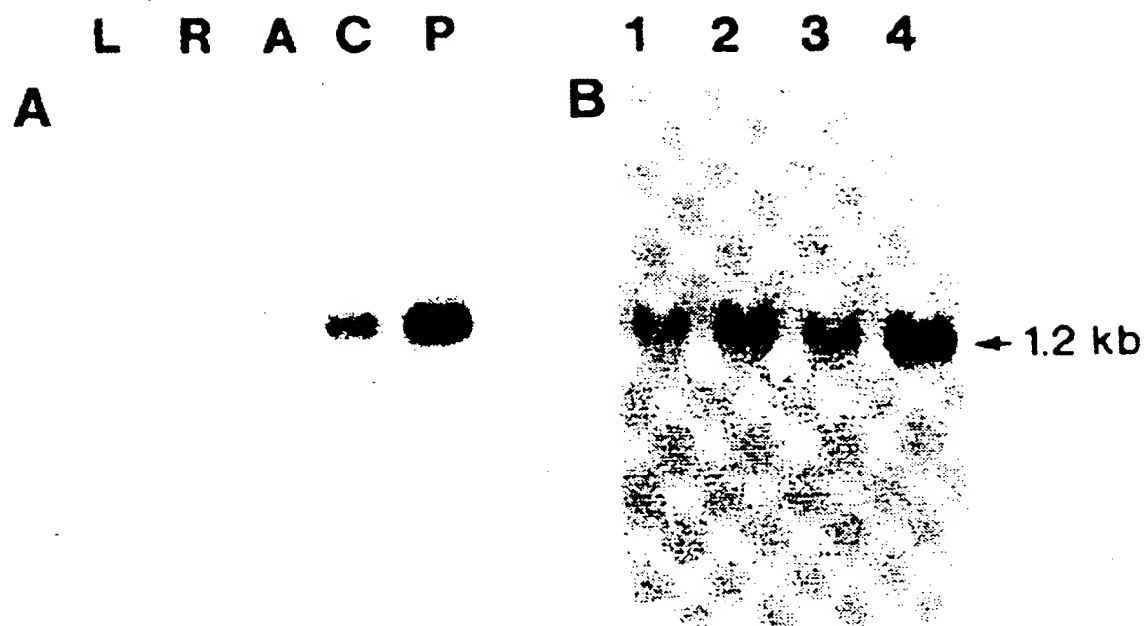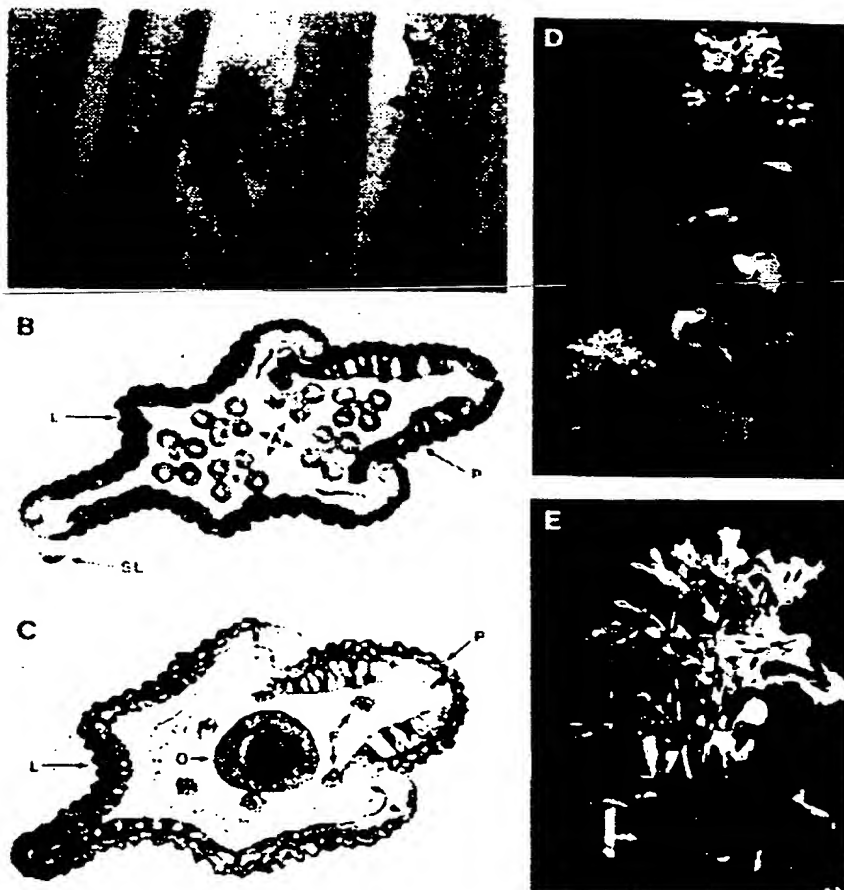
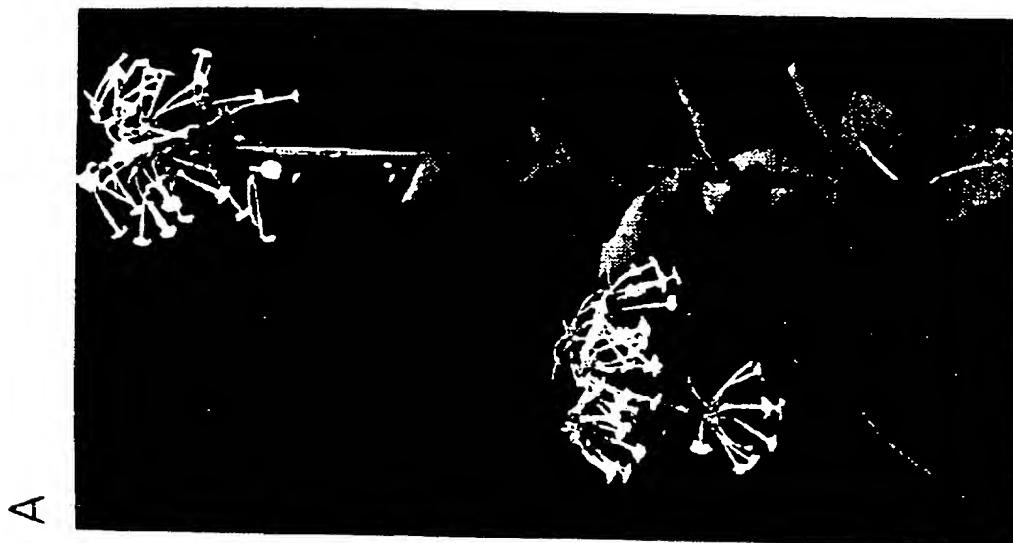FIGS. 2A-2B

FIGS. 3A-3B

FIGS. 4A-4E

FIG. 5

B

FIG. 6B

A

FIG. 6A
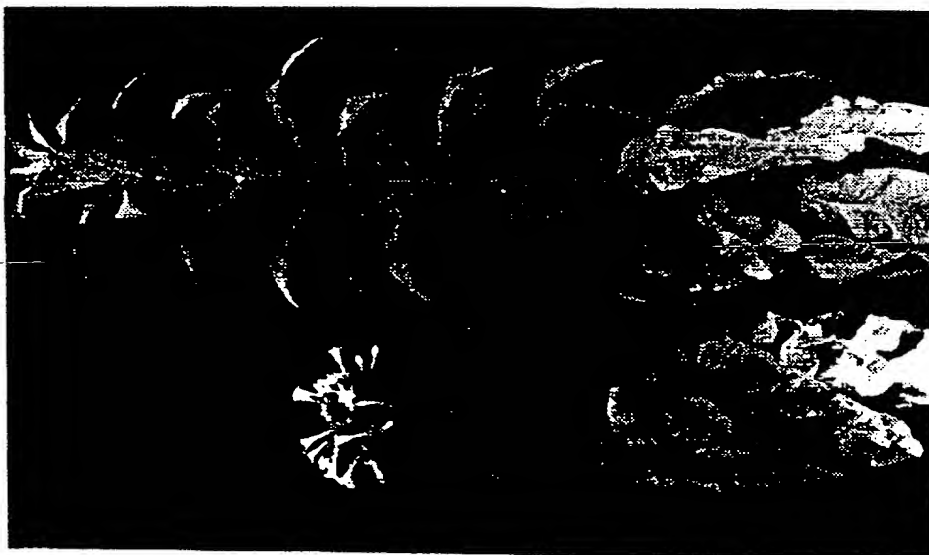
1 2 3 4 5 C

FIG. 7

FIG. 8B

FIG. 8A

1 2 3 C

FIG. 9

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  1 CCGGCCGCTGAAAAAATGGGAAGGGGTAGGGTTGAGCTTAAGAGAATAGAGAACAAGATC
                M  G  R  G  R  V  E  L  K  R  I  E  N  K  I   15
 61 AACAGGCAAGTGACCTTCGCTAAGAGAAGAAATGGACTTTTGAAAAAAGCTTATGAGCTT
       N  R  Q  V  T  F  A  K  R  R  N  G  L  L  K  K  A  Y  E  L   35
121 TCTGTTCTTTGTGATGCTGAGGTTGCTCTCATCATCTTCTCCAATAGGGGAAAACTGTAC
       S  V  L  C  D  A  E  V  A  L  I  I  F  S  N  R  G  K  L  Y   55
181 GAGTTCTGCAGTAGCTCTAGCATGCTCAAGACATTAGAGAGGTACCAGAAGTGCAACTAC
       E  F  C  S  S  S  M  L  K  T  L  E  R  Y  Q  K  C  N  Y   75
241 GGAGCACCAGAGACCAATATATCCACACGAGAAGCACTGGAAATAAGTAGCCAACAAGAA
       G  A  P  E  T  N  I  S  T  R  E  A  L  E  I  S  S  Q  Q  E   95
301 TACTTGAAGCTTAAAGCACGTTACGAAGCATTACAGCGATCACAGAGAAATCTTCTTGGT
       Y  L  K  L  K  A  R  Y  E  A  L  Q  R  S  Q  R  N  L  L  G  115
361 GAAGATCTTGGCCCCTTTGAATAGCAAGGAACTTGAATCACTTGAGAGGCAGCTTGATATG
       E  D  L  G  P  L  N  S  K  E  L  E  S  L  E  R  Q  L  D  M  135
421 TCACTGAAACAGATTCGATCAACTCGGACTCAGTTAATGTTGGATCAACTTACAGATCTT
       S  L  K  Q  I  R  S  T  R  T  Q  L  M  L  D  Q  L  T  D  L  155
481 CAGAGAAAGGAACATGCATTAAACGAAGCAAACAGAACCTTGAAACAAAGGTTGATGGAA
       Q  R  K  E  H  A  L  N  E  A  N  R  T  L  K  Q  R  L  M  E  175
541 GGAAGCCAACTAAATCTGCAGTGGCAACAAAATGCACAAGATATGGGCTACGGCCGGCAA
       G  S  Q  L  N  L  Q  W  Q  Q  N  A  Q  D  M  G  Y  G  R  Q  195
601 ACAACTCAAACTCAGGGCGATGGCTTTTTTCATCCTTTGGAATGTGAACCCACTTTGCAA
       T  T  Q  T  Q  G  D  G  F  F  H  P  L  E  C  E  P  T  L  Q  215
661 ATTGGGTATCAGAATGATCCAATAACAGTAGGAGGAGCAGGGCCCAGTGTGAATAACTAC
       I  G  Y  Q  N  D  P  I  T  V  G  G  A  G  P  S  V  N  N  Y  235
721 ATGGCTGGCTGGTTGCCTTGAAAATTAAGCTCATTTCCGATAAGATTGATTATATAAACAT
       M  A  G  W  L  P  *                                         241
781 ATGCTCAATGTTTTTCCTATCATAAACACTCTCCTAATTTGTGTTATATGTTGTTTGCCG
841 AATTCTGGACTAATTTGGGATCCATAAGACAGACCCGTTATTGTTACTTAATCATAAACT
886 AGATTTCCCTGAGTGACTAATCACTAAAGCTTATTACTTTCCTCC(A)
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FIG. 10

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  1 MGRGRVELKRIENKINRQVTFAKRRNGLLKKAYELSVLCDAEVALIIFSN 50
    ||||:|||||||||||.||||||||||||||||||||:||||||||||||.
  1 MGRGKVELKRIENKISRQVTFAKRRNGLLKKAYELSLLCDAEVALIIFSG 50

 51 RGKLYEFCSSSSMLKTLERYQKCNYGAPETNISTREALEISSQQEYLKLK 100
    ||:|:||:|||:|.||||||||..|||...:.. ...|    . |||||||
 51 RGRLFEFSSSSCMYKTLERYRSCNYNSQDAAAPENEI....NYQEYLKLK 96

101 ARYEALQRSQRNLLGEDLGPLNSKELESLERQLDMSLKQIRSTRTQLMLD 150
    .| | || .|||:|||||||||||. |||| ||.|::|||||||| :.| :||
 97 TRVEFLQTTQRNILGEDLGPLSMKELEQLENQIEVSLKQIRSRKNQALLD 146

151 QLTDLQRKEHALNEANRTLKQRLMEGSQ...LNLQWQQNAQDMG....YG 193
    || ||..||:.|.: |:.|:.:| |.|   |:: ||:.:.. |    .:
147 QLFDLKSKEQQLQDLNKDLRKKLQETSAENVLHMSWQDGGGHSGSSTVLA 196

194 RQTTQTQGDGFFHPLECEPTLQIGYQNDPI.........TVGGAGPSVNN 234
    |. : ||    || :.:..|||||::..       .:|:. |.:
197 DQPHHHQGLLHPHPDQGDHSLQIGYHHPHAHHHQAYMDHLSNEAADMVAH 246

235 YMAGWLP 241
    . .: :|
247 HPNEHIP 253
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FIG. 11

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A01H 5/00; C12N 5/10, 15/29, 15/82; C12P 21/02

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) r to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 69.1, 70.1, 172.3, 240.4, 320.1; 530/350; 536/23.6, 24.3; 800/205

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	THE PLANT CELL, Volume 5, issued September 1993, Matsuoka et al, "Expression of a Rice Homeobox Gene Causes Altered Morphology of Transgenic Plants", pages 1039-1048, see pages 1039, 1041-1042.	1-3,6-8,10-13,19 ---------------- 4,5,9, 14-18, 20-23
X -- Y	FEBS LETTERS, Volume 334, No. 3, issued November 1993, Kano-Murakami et al, "A Rice Homeotic Gene, OSH1, Causes Unusual Phenotypes in Transgenic Tobacco", pages 365-368, see pages 365-366.	1-3,6-8,10-13,19 ---------------- 4,5,9, 14-18, 20-23

[X] Further documents are listed in the continuation of Box C.   [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
04 JANUARY 1996	**31 JAN 1996**

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer DAVID T. FOX
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	MOLECULAR AND GENERAL GENETICS, Volume 239, No. 1-2, issued 1993, Hansen et al, "NTGLO: A Tobacco Homologue of the GLOBOSA Floral Homeotic Gene of Antirrhinum majus: cDNA Sequence and Expression Pattern", pages 310-312, see pages 310-311.	1-3,6-7,13 ------------ 4,5,8-12, 14-23
X --- Y	PLANT PHYSIOLOGY, Volume 103, No. 4, issued 1993, Kempin et al, "Conversion of Perianth into Reproductive Organs by Ectopic Expression of the Tobacco Floral Homeotic Gene NAG1", pages 1041-1046, see pages 1042 and 1044.	1-3,6-8,10-13 ------------ 4,5,9,14-23

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/6, 69.1, 70.1, 172.3, 240.4, 320.1; 530/350; 536/23.6, 24.3; 800/205

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